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PHENOTYPICAL CHARACTERISTICS OF WALNUT'S (*Juglans* sp.) BACTERIAL DISEASES AGENT

As known, walnut is vulnerable to various pathogenic microorganisms, in particular bacteria that can cause significant lesions during their cultivation. Over the last decade, causative agents of walnut's bacterial diseases, which previously did not affect this culture, have been discovered all over the world. The expansion of the spectrum of pathogens that can potentially affect walnuts can be associated with intense anthropogenic influence on the environment, changes in climatic conditions, globalization of the agricultural sector, and the selection of new varieties with a low content of tannins and alkaloids. The ***purpose*** *of the work is a comparative analysis of a complex of phenotype features of collection and isolated strains to identify the latter.* ***Methods.*** *Classic microbiological, API-testing, phytopathological, and biochemical methods were used in the research.* ***Results.*** *Strains of phytopathogenic bacteria were isolated from affected walnut tissues in pure culture and analyzed for a complex of morphological-cultural, physiological-biochemical, and pathogenic properties. All isolated strains are capable of infecting a certain set of indicator (carrot and popato explants, beans, tobacco) plants and host (walnut's) plants, have a stable biochemical profile, and are significantly related to representatives of Agrobacterium tumefaciens, Xanthomonas arboricola, and Pseudomonas syringae species. In the fatty acid profiles of isolated strains, there was detected a typical representative of species Agrobacterium tumefaciens, Xanthomonas arboricola, Pseudomonas syringae set of fatty acids.* ***Conclusions.*** *The phenotypic properties of bacterial isolated and collected strains causing walnut diseases were analyzed and their similarity to representatives of Agrobacterium tumefaciens, Xanthomonas arboricola, and Pseudomonas syringae was established. It should be noted that walnut leaf spotting is caused by Pseudomonas syringae, which was discovered by us for the first time in Ukraine.*

Keywords: *phenotypic properties, bacterial diseases of walnut, Pseudomonas syringae, Xanthomonas arboricola, Agrobacterium tumefaciens.*

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In the world, the most widespread species used for obtaining fruits are Persian (or English) walnut (*Juglans regia*), originating from Persia, and black walnut (*Juglans nigra*), originating from North America. In Ukraine, *Juglans regia* is the most common species, while *Juglans nigra* is the most common species found in the territory of the Right Bank Forest Steppe of Ukraine, and in particular in the forests of Vinnytsia region (Galushko & Berehovy, 2011). Traditionally, the cultivation of walnut in Ukraine was based on private households and forest belts, but recently the cultivation of this tree by an industrial method has increased significantly, which allowed our country in the pre-war period (2020—2021) to occupy the fifth place in the list of countries exporting this product (data from the USA Department of Agriculture). According to the literature, the high yield of the nut depends significantly on the absence of its lesions inducted by various pathogens. Despite the significant spread of walnut bacterial diseases, this problem has little studied in Ukraine (European and Mediterranean Plant Protection Organization; Patyka et al., 2017).

By the literature, the most harmful causative agent of walnut diseases is *X. arboricola* pv. *juglandis*. This pathogen can cause a decrease in nut yield by 40—60% (Giovanardi et al., 2015; Ilicic et al., 2021; Kim et al., 2021; Temperini et al., 2017; Yang et al., 1993). Recently, reports on walnut diseases caused by this pathogen have appeared all over the world (Giovanardi et al., 2015; Gasic et al., 2012; Kim et al., 2021; Osdaghi, 2022; Temperini et al., 2017). In particular, large-scale epiphytotia caused by this pathogen have recently been registered in: the northern province of North Gyeongbuk, Korea (2021) (Kim et al., 2021), Serbia (2019—2020) (Ilicic et al., 2021), in the northern part of Argentine Patagonia (2013—2014) (Temperini et al., 2017), the Romagna region of Italy (2007—2010) (Giovanardi et al., 2015), etc. It should be noted that this pathogen was first isolated in Argentine Patagonia (Temperini et al., 2017) and Serbia (Ilicic et al., 2021). In addition, in Argentine Patagonia, it was *X. arboricola* pv. *juglandis* that accounted for the largest percentage (66.7—53.3%) of mixed infections causing damage to walnut plantations (Temperini et al., 2017). Researchers also note that *X. arboricola* pv. *juglandis*, unlike fungal pathogens, penetrates the deepest into the internal tissues of fruits, there by causing their greatest damage (Temperini et al., 2017). The scientific community responded to the significant harmfulness and rapid spread of this pathogen by creating a special section dedicated to the comprehensive study of *X. arboricola* pv. *juglandis* in the leading specialized database (CABI) (Osdaghi, 2022). Researchers state that the reasons for the rapid spread of this pathogen are an increase in the production of planting material and its mass import/export to different countries, the breeding of varieties that are sensitive to *X. arboricola* pv. *juglandis*, and changing climatic conditions (Yang et al., 2021). In addition, some scientists note that this pathogen can remain for a long time as an epiphyte under the scales of dormant buds without causing symptoms of the disease until the onset of a susceptible moment for pathogen invasion (Temperini et al., 2017). In 2012, massive walnut leaf spotting was detected in the Hamedan province in Iran (Keshtkar, 2016). Based on the complex of phenotype and genotype features, the pathogen was identified as *P. syringae* pv. *syringae*. This is the first report on walnut's lesion by *P. syringae* pv. *syringae* in the world. As known, this species is a classic polyphage and is capable of affecting a wide range of host plants. In particular, Iranian researchers note that strains isolated from walnut were pathogenic for peach and plum trees (Keshtkar et al., 2016). In addition to *X. arboricola* pv. *juglandis, P. syringae* pv. *syringae* are able to remain as an epiphyte on plants for a long time and withstand low temperatures, which greatly contributes to their spread (Giovanardi et al., 2015; Ilicic et al., 2021; Popović et al., 2021; Zarei et al., 2019). In 2009—2010, researchers from Iran registered epiphytotia in walnut plantations caused by *A. tumefaciens* (Rouhrazi and Rahimian, 2014). This pathogen

caused crown gall of the walnut's root in southern Iran. As known, *A. tumefaciens* is a classical polyphage and is capable of inducing tumor forming in parent cultures (Rouhrazi & Rahimian, 2014; Tekiner & Kotan, 2022). It also overwinters in soil and infected plant material, which somewhat facilitates its spread. A number of researchers have also described the ability of bacteria of the genus *Brenneria* to cause walnut diseases (European and Mediterranean Plant Protection Organization, 2024). In particular, the causative agent of deep bark cancer is *Brenneria rubifaciens*, and *Brenneria nigrifluens* is the causative agent of shallow bark cancer. Recently, Serbian scientists for the first time established the presence of this pathogen in walnut plantations. However, they emphasize that the number of tree lesions caused by this pathogen is significantly lower than that of *X. arboricola* pv. *juglandis* (Giovanardi et al., 2015). Researchers also note the ability of the pathogen to be in an epiphytic state for many years before the plant shows the disease symptoms. Because most of the pathogens described above are able to remain for a long period in an epiphytic state on walnut trees and also survive under adverse climatic conditions, timely and correct diagnosis and identification of them are extremely relevant and important (Giovanardi et al., 2015). One of the key aspects of accurate diagnosis and identification of pathogens is the analysis of their phenotype properties (Brenner et al., 2005; Dankevich et al., 2016). Accoding to the literature, usually, the study of pathogenic, morphological, and cultural properties and utilization of some substrates as the only source of nutrition is the basis of phenotype identification of these pathogens (Dankevich et al., 2016). In addition, it is known that the fatty acid composition of cellular lipids is an important taxonomic feature for the identification of phytopathogenic bacteria (Stead, 1992; Stead et al., 1992).

Therefore the **aim** of our research was to analyze a complex of phenotype features of both isolated and collected strains capable of affecting walnut trees.

Materials and Methods. In this study, we used strains isolated from affected walnut trees such as: *Agrobacterium* spp. — 1gor, 3gor, 8gor; *Xanthomonas* spp. — 1or, 3or; *Pseudomonas* spp. — 4n, 5n. For comparative analysis, collection and typical strains of species that, according to the literature, can affect walnuts were included in the study, namely, *A. tumefaciens* — 8466, 8464, 8461, 8460, 8462, 8465, 8428, 8469, 8463, 8467, 9054, 9053, 8933^{T}; *X. arboricola* pv. *juglandis* — 8665, 8666, 8865, *P. syringae* pv. *syringae* — UCM B-1027^{T}. The selection of affected aerial parts and roots of walnut trees was carried out in different climate-geographical regions on the territory of the Mykolaiv (steppe) and Kyiv regions (mixed forests) of Ukraine in the period from May to mid-July at 2020-2021. Isolation of bacterial isolates from affected walnut tissues was carried out by generally accepted methods (Patyka et al., 2017). Pathogenic properties of pure isolated cultures of bacteria were studied by the generally accepted method of artificial infection of indicator plants and host plants. The strain's aggressiveness was assessed on the 4—10th day after infection, according to a common 5-point scale. (Patyka et al., 2017). The ability of *Agrobacterium* sp. isolated and collection strains to induct tumor formation was checked on carrot, and potato explants, and Kalanchoe leaf using known methods (Patyka et al., 2017). The hypersensitivity reaction caused by pathogenic bacteria of the genus *Pseudomonas* was carried out on tobacco leaves by a common method. The cultural and physiological properties of bacteria were studied as described by Patyka et al. (Patyka et al., 2017). The physiological and biochemical properties of bacteria were studied using API test systems 20E bioMérieux (France) bacterial identification kits, according to the manufacturer's instructions.

For the study of cellular lipids fatty asids composition, bacteria were grown on potato dextrose agar (24 hours). The one-day culture was washed off with physiological solution, precipitated by centrifugation for 40—60 min at 1500 rev min^{-1}. For the study 10 mg of cells were used in the count of dry weight of bacteria. The bacterial cells

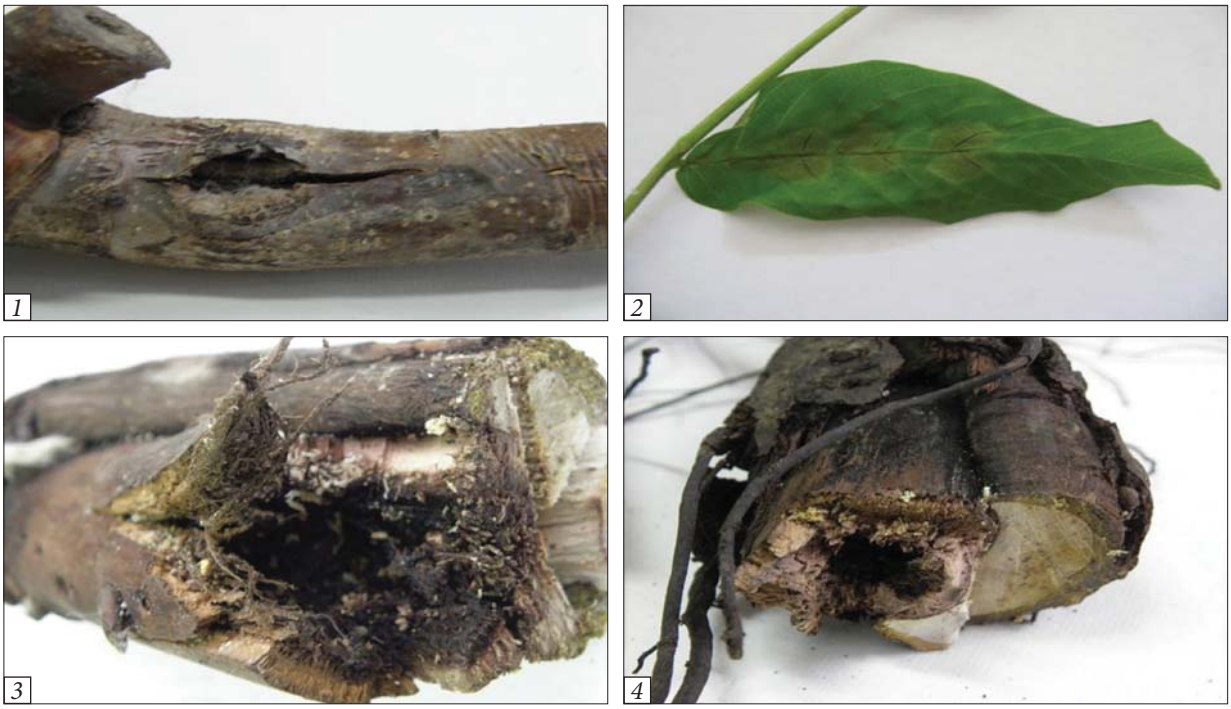

Fig. 1. Symptoms of walnut's lesion caused by bacterial pathogens: *1* — stem; *2* — leaf surface; *3, 4* — root system

were kept in a 5% acetyl chloride in methanol solution for 4 hours at 100 °C, and 2—3 mL of distilled water was added later. The fatty acid methyl esters were extracted twice with an ether/hexane mixture (1:1) (Dankevich et al., 2016; Patyka et al., 2017). The composition of fatty acid methyl esters was analyzed using a gas chromatography-mass spectrometry system Agilent 6800N/5973 inert. Methyl esters were identified automatically by their retention time compared to standards. Fatty acid content was determined using Agilent ChemStation software and displayed as % of the total peak area. To assess the reliability of the experimental data presented in the paper, the parametric criteria of normal distribution were used in calculating the arithmetic mean (Xmean) and the mean square deviation (SXmean) at a significance level of <0.05. The analysis was carried out using the package of computer programs STATISTICA 6.0 and Microsoft Excel.

Results. Walnut lesions caused by bacterial pathogens on samples of the affected tissues of the root, leaves, fruits, and trunk were analyzed. From affected tissues, bacterial cultures were isolated, selected, and analyzed (Fig. 1, Fig. 2).

According to the results of the analysis of pathogenic, morphological-cultural, and physiological-biochemical properties, isolated strains of bacteria pathogenic for walnut were divided into three groups (Table 1).

The first group of isolated strains includes are yellow, smooth, mucous colonies which may become somewhat rough over time (Fig. 2). Under the microscope, these are gram-negative, small rods, mobile due to one polar flagellum. They don't produce pigment in the cultural medium, metabolize glucose according to the oxidative type, are variable in their ability to hydrolyze starch.

Don't reduce nitrates. Gelatin is diluted, and milk is coagulated (peptonized) (Table 1). Able to produce catalase, β-galactosidase, but do not synthesize oxidase and a number of other enzymes (Table 2). They do not produce indoles, H_2S, and acetoin and don't utilize citrates (Table 2).

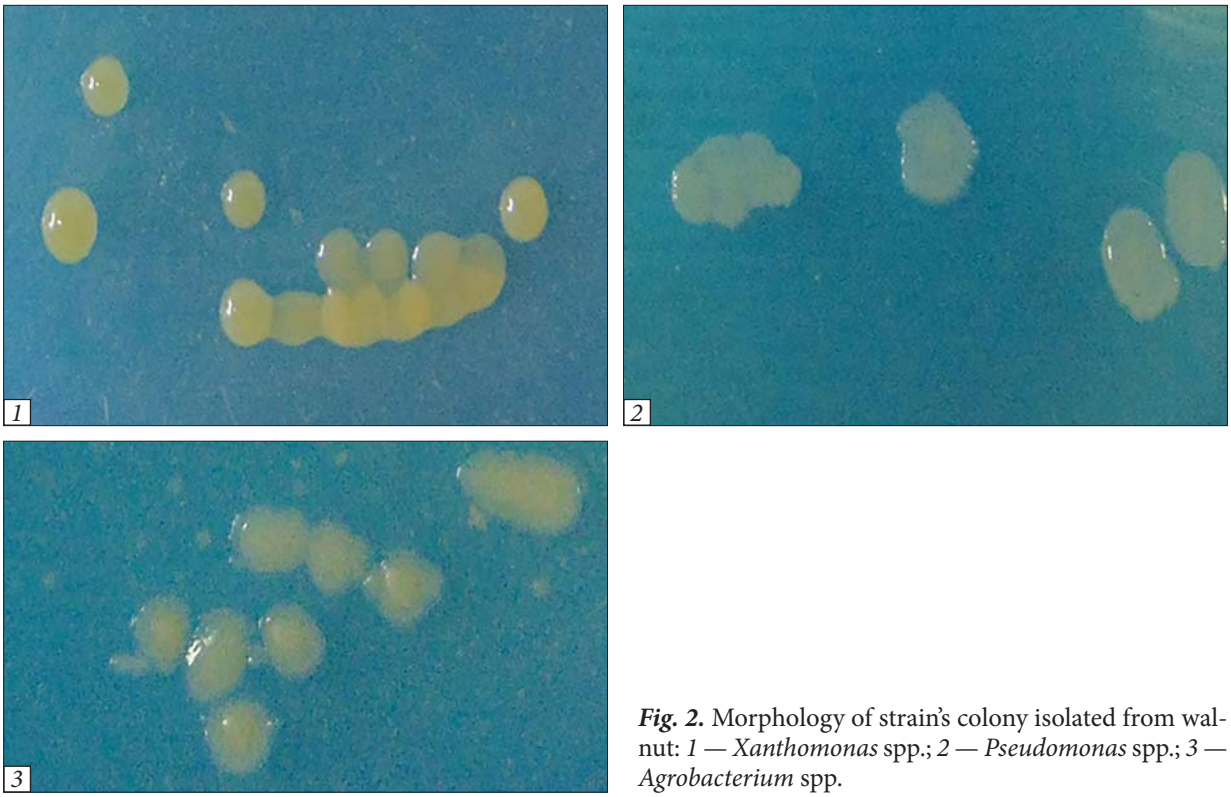

Fig. 2. Morphology of strain's colony isolated from walnut: *1 — Xanthomonas* spp.; *2 — Pseudomonas* spp.; *3 — Agrobacterium* spp.

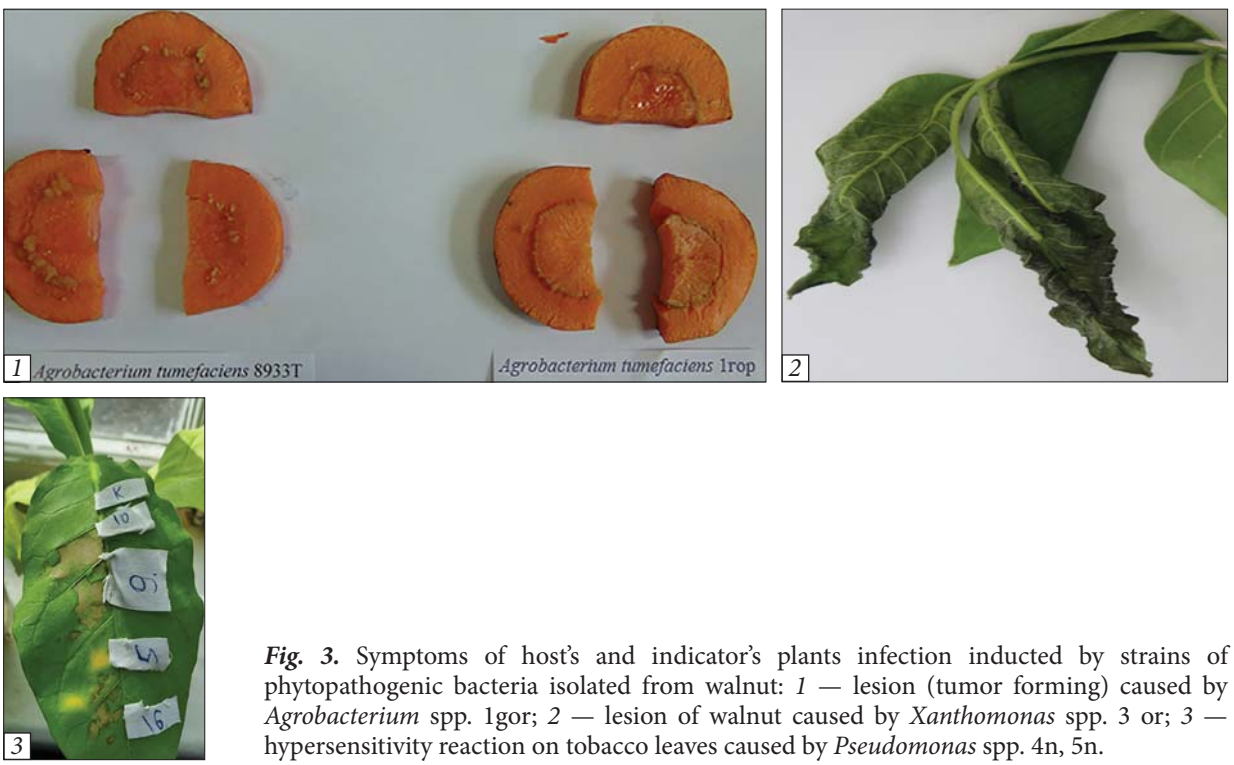

Fig. 3. Symptoms of host's and indicator's plants infection inducted by strains of phytopathogenic bacteria isolated from walnut: *1* — lesion (tumor forming) caused by *Agrobacterium* spp. 1gor; *2* — lesion of walnut caused by *Xanthomonas* spp. 3 or; *3* — hypersensitivity reaction on tobacco leaves caused by *Pseudomonas* spp. 4n, 5n.

Table 1. **Physiological and biochemical properties of the studied strains**

Property	*Pseudomonas* spp.	*P. syringae* pv. *syringae* B1027^T	*Xanthomonas* spp.	*X. arboricola* pv. *juglandis* collection strains	*Agrobacterium* spp.	*A. tumefaciens* collection strains
Gram staining	—	—	—	—	—	—
Mobility	+	+	+	+	+	+
Production of indole, H_2S	—	—	—	—	—	—
Oxidase production	—	—	—	—	+	+
Catalase production	+	+	+	+	+	+
Nitrate reduction	+	+	—	—	—	—
Milk coagulation	+	+	+	+	+	+
Gelatin dilution	+	+	+	+	—	—
Litmus serum	al	al	al	al	al	al

(—) — positive reaction; (+) — negative reaction; (al) — fermentation of substances with the formation of alkali.

Table 2. **Physiological and biochemical properties of phytopathogenic bacteria isolated from affected walnut tissues and collection strains**

Reaction/enzyme, Fermentation/oxidation.	Genus, species, strain of microorganisms					
	P. s. pv. *syringae* UCM B-1027^T	*Pseudomonas* spp.	*X. arboricola* pv. *juglandis*	*Xanthomonas* spp.	*A. tumefaciens*	*Agrobacterium* spp.
Formation, synthesis						
β-galactosidase	—	—	+	+	+	+
Argininedihydrolase	—	—	—	—	+/—	+/—
Lysinedecarboxylase, Ornithinedecarboxylase	—	—	—	—	—	—
Urease, acetoin	—	—	—	—	+	+
Tryptophan deaminase	+	+	—	—	+	+
Citrate utilization	+	+	—	—	—	—
Fermentation/oxidation						
D-glucose, N_2 production	+	+	+	+	+	+
D-mannitol, inositol, D-sorbitol, D-melobiose, amygdalin	—	—	—	—	—	—
L-rhamnose	—	—	—	—	+/—	+/—
D-saccharose	+	+	—	—	—	—
L-arabinose	+	+	—	—	+/—	+/—
NO_2 production	—	—	—	—	—	—

(—) — positive reaction; (+) — negative reaction; (al) — fermentation of substances with the formation of alkali.

The strains belonging to this group inducted the hypersensitivity reaction on bean leaves and necrosis on walnut leaves (Fig. 3, Table 3).

The properties of the above-listed isolated strains that listed above, correlate with the similar ones of the collection strains, which indicates their affinity with representatives of the genus *Xanthomonas*.

The second group of isolated strains formed blue-gray translucent colonies with a smooth or slightly wavy edge on solid agar media (Fig. 2). The center of the colony is slightly raised. The colonies form a fluorescent pigment. Gram-negative, mobile due to one or more polar flagella. Does not reduce nitrates. Gelatin is diluted, and peptonized milk is curdled (Table 1). Able to produce catalase, tryptophan deaminase, and citrates but do not produce β-galactosidase, oxidase, and some other enzymes and do not produce indoles, H_2S, and acetoin. Able to ferment D-glucose, D-sucrose, and L-arabinose and produce acid (Table 2). Related to representatives of the species *P. syringae* pv. *syringae* by the LOPAT test. They induce a hypersensitivity reaction on tobacco leaves, which indicate their affinity to typical representatives of the genus *Pseudomonas*, in particular to the typical strain of *P. syringae* pv. *syringae* UCM B-1027^T (Table 3, Fig. 3).

The third group of isolated strains formed raised moist shiny cream colonies with even edges when seeded on solid nutrient media (Fig. 2). These are gram-negative, small rods, and mobile due to one flagellum (Table 1). Able to synthesize catalase, oxidase, urease, and tryptophan deaminase. Capable of fermenting glucose and variable in their ability to synthesize L-rhamnose and L-arabinose (Table 2). After infection of potatoes and carrots explants, as well as Kalanchoe leaves, after 14—24 days, they are able to induce intensive tumor formation, which makes them similar to typical representatives of the *Agrobacterium tumefaciens* species (Table 3, Fig. 3).

Chemotaxonomic analysis based on the study of the fatty acid composition of cellular lipids is important for species definition (Dankevich et al, 2016; Stead, 1992). In the fatty acid spectra of isolated *Xanthomonas* spp. and collection *X. arboricola* pv. *juglandis* strains, fatty acids with a carbon chain length from C_{12} to C_{18} were found, namely: saturated acids— decanoic ($C_{10:0}$), undecanoic ($C_{11:0}$), tetradecanoic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$) acids; unsaturated acids — hexa- ($C_{16:1}$) and octadecenoic ($C_{18:1}$); hydroxy — 3-hydroxydecanoic (3-OH-$C_{10:0}$), 2-hydroxydecanoic (2-OH-$C_{10:0}$),

Table 3. **Pathogenic properties of strains isolated from walnut and collection strains**

Test	*Pseudomonas* spp. isolated strains 4n,5n	*P. syringae* pv. *syringae* B1027^T	*Xanthomonas* spp. isolated strains 1or, 3or	*X. arboricola* pv. *juglandis* collection strains 8665-8865	*Agrobacterium* spp. isolated strains 1 gor, 3 gor, 8 gor	*A. tumefaciens* collection strains 8466-8933^T
Induction of tumorigenesis on carrot and potato explants	—	—	—	—	+	+
Induction of a hypersensitivity reaction to tobacco leavs (HR)	+	+	—	—	—	—
Induction of a hypersensitivity reaction on bean	+	+	+	+	—	—
The ability to affect leaves, branches, nuts of walnut	+	+	+	+	—	—

(—) — positive reaction; (+) — negative reaction; (al) — fermentation of substances with the formation of alkali.

3-hydroxydodecanoic (3-OH-$C_{12:0}$), 2-hydroxydodecanoic (2-OH-$C_{12:0}$), 3-hydroxytetradecanoic (3-OH-$C_{14:0}$), 3-hydroxyhexadecanoic (3-OH-$C_{16:0}$). In the fatty acid spectra of isolated *Xanthomonas* spp. and collection strains of *X. arboricola* pv. *juglandis*, a wide range of iso and anteiso forms of fatty acids were also detected, in particular: 13-methyl tetradecanoic ($C_{15:0iso}$), 12-methyl tetradecanoic ($C_{15:0anteiso}$), 14-methyl pentadecanoic ($C_{16:0\ iso}$), 15-methyl hexadecanoic ($C_{17:0\ iso}$), 14-methyl hexadecanoic ($C_{17:0\ anteiso}$). In addition, cyclopropane fatty acid with 17 carbon atoms was detected in the fatty acid spectra of cellular lipids.

It was found that in the largest quantities in the cellular lipids of the collection *X. arboricola* pv. *juglandis* and isolated *Xanthomonas* spp. strains are hexadecanoic ($C_{16:0}$) from 31.35% to 33.55%, hexadecenoic ($C_{16:1}$) from 19.2% to 22.75%, and 12-methyl tetradecanoic ($C_{15:0anteiso}$) from 11.98% to 13.10% acids, which is a characteristic feature of representatives of the genus *Xanthomonas* (Yang et al., 1993). Therefore, the fatty acid spectra of the collection and isolated from samples of affected walnut tissues strains are significantly related. This indicates a high probability that the isolated strains belong to the species *X. arboricola* pv. *juglandis*.

In the fatty acid profiles of *Pseudomonas* spp strains isolated from walnut and the typical strain of *P. syringae* pv. *syringae* — UCM B-1027^T, the following fatty acids with a carbon chain length from C_{10} to C_{19} were found: unsaturated — hexa- ($C_{16:1}$) and octadecenoic ($C_{18:1}$); saturated — decanoic ($C_{10:0}$), dodecanoic ($C_{12:\ 0}$), tetradecanoic ($C_{14:0}$), hexadecanoic ($C_{16:0}$), and octadecanoic ($C_{18:0}$) ; hydroxy — 3- hydroxydecanoic (3-OH-$C_{10:0}$), 2-hydroxydodecanoic (2-OH-$C_{12:0}$), 3-hydroxydodecanoic (3-OH-$C_{12:0}$), and cyclopropane acids with 17 and 19 carbon atoms. In the largest amounts in cell lipids of typical *P. syringae* pv. *syringae* UCM B-1027^T and isolated strains of *Pseudomonas* spp. were present: cis-9 hexadecenoic ($C_{16:1cis9}$) acid from 34.58 to 35.79%; cis-9 octadecenoic acid ($C_{18:1cis9}$) from 22.78% to 23.59%, and trans hexadecenoic acid ($C_{16:1trans}$)

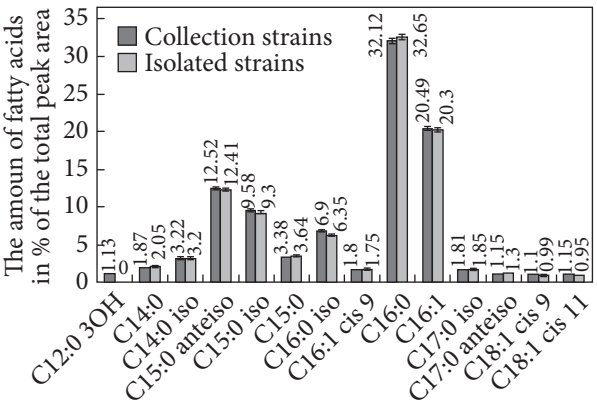

Fig. 4. Cellular lipids fatty acid composition of collection *X. arboricola* pv. *juglandis* and isolated from affected walnut tissues *Xanthomonas* spp. strains

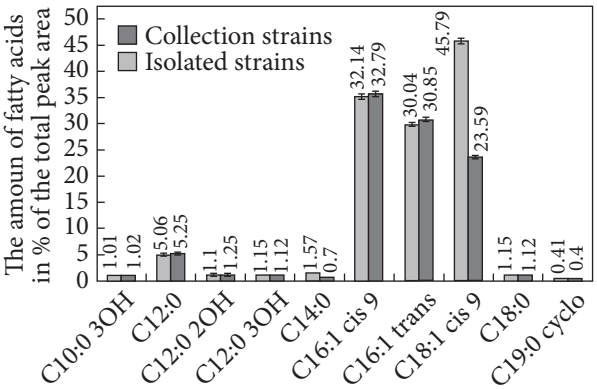

Fig. 5. Cellular lipids fatty acid composition of typical *P. syringae* pv. *syringae* UCM B-1027^T and isolated from affected walnut tissues *Pseudomonas* spp. strains

from 29.98% to 30.85%, respectively. Acording to literature, the most important for the taxonomy of *P. syringae* bacteria is the presence of hydroxyl fatty acids (Stead, 1992), 3-oxidodecanoic (3-OH—$C_{10:0}$), 2-oxidodecanoic (2-OH—$C_{12:0}$), and 3-oxidodecanoic (3-OH—$C_{12:0}$) acids. These fatty acids were contained in amounts from 0.98% to 1.25%. Characterization of the cellular lipids fatty acid profiles established the quantitative and qualitative relationship between the *Pseudomonas* spp. strains isolated by us from walnut tissues and the typical strain of *P. syringae* pv. *syringae* UCM B-1027^T.

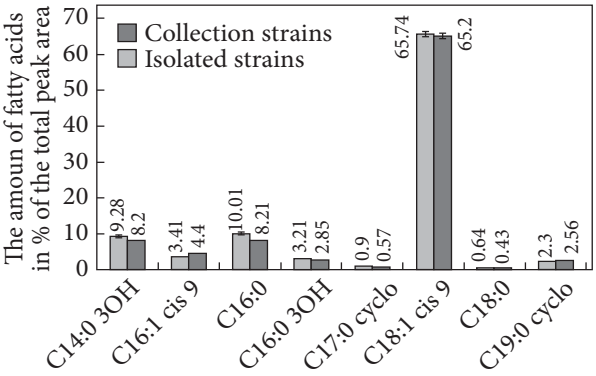

Fig. 6. Cellular lipids fatty acid composition of collection *A. tumefaciens* and isolated from affected walnut tissues *Agrobacterium* spp. strains

The cellular lipids of *A. tumefaciens* collection and isolated strains are characterized by the presence of fatty acids with a carbon chain length from C_{14} to C_{19}, namely: saturated — hexadecanoic ($C_{16:0}$), octadecanoic ($C_{18:0}$); unsaturated — hexa- ($C_{16:1}$) and octadecenoic ($C_{18:1}$); hydroxyl — 3-hydroxydecanoic (3-OH-$C_{10:0}$), 3-hydroxytetradecanoic (3-OH-$C_{14:0}$), 3-hydrohexadecanoic (3-OH-$C_{16:0}$) and cyclopropane acids with 17 ($C_{17:0\ cyclo}$) and 19 ($C_{19:0cyclo}$) carbon atoms. In the largest quantities in the cell lipids of the collection *A. tumefaciens* and isolated *Agrobacterium* spp. strains, cis-9 octadecenoic ($C_{16:1cis9}$) acid from 46.42% to 77.12% is present. It is also worth noting that the amount of hexadecanoic acid ($C_{16:0}$) ranges from 7.89% to 11.87%. Cis-9,10-methylene hexadecanoic ($C_{17:0cyclo}$) and octadecanoic ($C_{18:0}$) acids were found as minor components (from 0.5 to 1%). It should be emphasized that the fatty acid profiles of *Agrobacterium* spp. collected and isolated from walnut strains are similar. This fact indicates the relationships of the isolated strains with representatives of the species *A. tumefaciens*.

Therefore, according to the complex of phenotype characteristics, the isolated from walnut strains of phytopathogenic bacteria are related to the typical representatives of the species *A. tumefaciens*, *X. arboricola* pv. *juglandis*, and *P. syringae* pv. *syringae*.

Discussion. In Ukraine, walnut bacterial diseases are not sufficiently studied and are a threat to walnut cultivation and significant decrease in its yield (Osdaghi, 2022). This work is the first step toward a better understanding of the spectrum and distribution of the causative agents of walnut bacterial diseases in Ukraine. According to our preliminary results in the Mykolaiv region, epiphytophyta of walnut plantations caused by *A. tumefaciens* was detected (unpublished data). Besides, it was possible to isolate *P. syringae* pv. *syringae* and *X. arboricola* pv. *juglandis* from samples of affected walnut tissues. It should also be emphasized that lesions to walnut leaves caused by *P. syringae* pv. *syringae* were registered on the territory of Ukraine for the first time (European and Mediterranean Plant Protection Organization, 2024). We previously determined the ability of these strains to synthesize the species-specific toxin syringomycin using biotesting and PCR (Dankevich & Zarudnyak, 2023).

It is also interesting that *A. tumefaciens*, which is able to overwinter in the soil, was found in a warmer climate region. Instead, *P. syringae* pv. *syringae* and *X. arboricola* pv. *juglandis*, which can withstand low temperatures (Gvozdyak et al., 2011) are isolated in the northern part of our country. All isolated pathogens were the only cause of the disease. On the other hand, according to the literature, causative agents of bacterial diseases of walnut are often found as part of a mixed infection (Giovanardi et al., 2015). In the case of diagnosing *A. tumefaciens*, the disease manifestation didn't occur immediately from the moment of infection, but was in a latent form. In our case, the pathogen was introduced in Ukraine with planting material from abroad. That is why many studies are devoted to the timely detection, diagnosis, and identification of phytopathogenic bacteria (Dankevich et al., 2016; Dankevich & Zarudnyak, 2023; European and Mediterranean Plant Protection Organization, 2024; Galushko & Berehovy, 2011; Gasic et al., 2012). The study of the phenotype of bacterial pathogens begins with the study of their pathogenic properties (Galushko & Berehovy, 2011; Patyka et al., 2017). Usually, in the case of testing the pathogenic properties of *X. arboricola* pv. *jug-*

landis infected leaves and fruits of walnut, a hypersensitivity reaction on bean leaves and pods is used (Kim et al., 2021). The pathogenic properties of *A. tumefaciens* are studied on potato, carrot, and zucchini explants, and by infecting Kalanchoe leaves, tomato seedlings, etc. (Rouhrazi & Rahimian, 2014). Pathogenicity of *P. syringae* pv. *syringae* was studied using a hypersensitivity reaction on tobacco leaves, infection of lilac or host plant (Patyka et al., 2017; Osdaghi, 2022). As it known, the populations of the causative agents of walnut's bacterial diseases show different variability of morphological-cultural and physiological-biochemical properties. In particular, some researchers note that *X. arboricola* pv. *juglandis* and *A. tumefaciens* have a stable metabolic profile (Bouza et al., 1993; Kim et al., 2021). For example, the strain's similarity with typical representatives of *X. arboricola* pv. *juglandis,* according to the results of biochemical reactions Vitex 2, Biolog, is 94—99% (Kim et al., 2021). Instead, *P. syringae* pv. *syringae* is somewhat more variable by this properties (Galushko & Berehovy, 2011; Popović et al., 2021).

The fatty acid composition of cellular lipids is an important chemotaxonomic marker in bacterial identification. In particular, it is known that the representatives of *P. syringae* pv. *syringae* is characterized by the presence of 3-hydroxydecanoic (3-OH—$C_{10:0}$), 2-hydroxydodecanoic (2-OH—$C_{12:0}$), and 3-hydroxydodecanoic (3-OH—$C_{12:0}$) acids. D. E. Stead established a rule for all phytopathogens belonging to the *Pseudomonas syringae* species (2-OH—$C_{12:0}$ less than 3% of the total peak area, $C_{16:1}$ +$C_{18:1}$ more than 52%, ratio $C_{16:0}$ to $C_{16:1}$ less than 0.9), which is reliable for all fatty acid profiles of isolated *Pseudomonas* spp strains (Stead et al., 1992). By the literature, 99% of 966 tested bacterial strains of the *Xanthomonas* genus have a large number of iso and anteiso fatty acids in the profilies. This feature is characteristic of all bacteria of this genus (Brenner et al., 2005; Yang et al., 1993). In particular, the presence of $C_{15:0iso}$ and $C_{15:0anteiso}$, $C_{17:0iso}$ and $C_{15:0anteiso}$ in the fatty acid spectra is a characteristic feature of many representatives of the genus *Xanthomonas*. According to the cluster analysis, bacteria of the species *X. albilineans*, *X. axonopodis*, *X. fragariae*, *X. maltophilia,* and *X. populi* are related by fatty acid cellular lipids profiles. According to some researchers, $C_{10:0}$, $C_{10:0\ 2OH}$, $C_{11:0\ 3OH}$, $C_{12:0}$, $C_{13:0iso}$, $C_{13:02OH}$, $C_{17:1\ \omega6c}$, $C_{17:110\text{-methyl,}}$ and $C_{17:03OH}$ fatty acids were less than 1.22% of the total peak area or absent in all strains (Zarei et al., 2019), which is consistent with our results. By the literature, representatives of the genus *Agrobacterium* are characterized by the presence of the following fatty acids in their profiles: hexadecanoic ($C_{16:0}$), 3-oxyhexadecanoic (3-OH—$C_{16:0}$), and cyclopropane fatty acid with 19 carbon atoms ($C_{19:0cyclo}$) (Bouza, et al., 1993). In addition, the presence of cyclopropane fatty acid with 17 ($C_{17:0cyclo}$) carbon atoms in cellular lipids may indicate a relationship with representatives of biovar I of this species (Bouza, et al., 1993; Tighe et al., 2000).

Conclusions. Pure cultures were separated from a walnut's lesion, and a complex of its morphological, cultural, physiological, and biochemical properties was analyzed as well as a chemotaxonomic analysis of the cellular fatty acid composition. The comparative analysis of the phenotypical properties of strains isolated from affected walnut's tissues revealed their close similarity with those of representatives of the *P. syringae* pv. *syringae*, *A. tumefaciens,* and *X. arboricola* pv. *juglandis*. It should be emphasized that a walnut lesion by *P. syringae* was discovered for the first time in Ukraine. Of course, to more correctly determinate walnut's bacterial diseases agents species, it is necessary to study their genotypic properties, which we plan to do in the future.

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ФЕНОТИПОВІ ВЛАСТИВОСТІ ЗБУДНИКІВ
БАКТЕРІАЛЬНИХ ХВОРОБ ГОРІХА ВОЛОСЬКОГО (*Juglans* sp.).

Як відомо, горіх волоський вразливий до різних патогенних мікроорганізмів, зокрема бактерій, які можуть завдати значних збитків при його вирощуванні. За останнє десятиліття у різних регіонах світу було виявлено збудників бактеріальних хвороб горіха волоського, що раніше не уражували цю культуру. Розширення спектру збудників, що потенційно можуть уражувати горіх волоський, може бути пов'язано з інтенсивним антропогенним навантаженням на довкілля, зміною кліматичних умов, глобалізацією аграрного сектору і селекцією нових сортів з низьким вмістом дубильних та алкалоїдних речовин. **Метою** роботи є порівняльний аналіз комплексу ознак фенотипу колекційних та ізольованих штамів з метою ідентифікації останніх. **Методи.** У дослідженнях використали класичні мікробіологічні, API-тестування, фітопатологічні та біохімічні методи. **Результати.** З уражених тканин горіха волоського виділено в чисту культуру штами фітопатогенних бактерій та проаналізовано їх за комплексом морфолого-культуральних, фізіолого-біохімічних та патогенних властивостей. Усі ізольовані нами штами здатні інфікувати певний набір індикаторних рослин (експланти моркви і картоплі, боби) та рослини-господаря (горіх волоський), мають стабільний біохімічний профіль та значно споріднені з представниками видів *Agrobacterium tumefaciens*, *Xanthomonas arboricola, Pseudomonas syringae*. У жирнокислотних профілях клітинних ліпідів ізольованих штамів виявлено типовий для представників видів *Agrobacterium tumefaciens*, *Xanthomonas arboricola, Pseudomonas syringae* набір жирних кислот. **Висновки.** Проаналізовано фенотипові властивості ізольованих і колекційних штамів бактерій, що викликають хвороби горіху волоського та встановлено їх спорідненість з представниками *Agrobacterium tumefaciens, Xanthomonas arboricola* та *Pseudomonas syringae.* Слід відмітити, що плямистість листя горіху волоського, викликана *Pseudomonas syringae*, виявлена нами вперше на території України.

Ключові слова: *фенотипові властивості, бактеріальні хвороби горіху волоського, Pseudomonas syringae, Xanthomonas arboricola, Agrobacterium tumefaciens.*